

THAT WHICH IS CLAIMED:

A method for determining the ability of a compound to affect the differentiation of preadipocytes to adipocytes, comprising:

- a) plating isolated human preadipocyte cells at a density of about 25,000 to 40,000 cells/cm<sup>2</sup> in a preadipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose;
- b) incubating said cells at about 37°C for about 4-48 hours until said cells are about 95-100% confluent;
- c) replacing said preadipocyte medium with a differentiation medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; a cyclic AMP inducer; 100 nM to 1 µM insulin, or an equivalent amount of an insulin analogue; 16 nM to 1 µM of a glucocorticoid; and said compound in an appropriate vehicle or vehicle alone;
- d) incubating said cells at about 37°C for about 2-4 days;
- e) replacing said differentiation medium with an adipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; 100 nM to 1 µM insulin, or an equivalent amount of an insulin analogue; and 16 nM to 1 µM of a glucocorticoid;
- f) incubating said cells at about 37°C for about 1-2 weeks and refeeding said cells with said adipocyte medium at least every 3-4 days; and
- g) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with said differentiation medium containing said compound;
- h) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with said differentiation medium containing said vehicle alone; and
- i) comparing the number or percentage of differentiated cells from steps (g) and (h);

wherein said preadipocyte medium, said differentiation medium and said adipocyte medium are maintained at a pH of about 7.0 to 7.6 when in contact with said cells.

✓ A method for determining the ability of a compound to act as a PPAR $\gamma$  antagonist, comprising:

- a) plating isolated human preadipocyte cells at a density of about 25,000 to 40,000 cells/cm<sup>2</sup> in a preadipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose;
- b) incubating said cells at about 37°C for about 4-48 hours until said cells are about 95-100% confluent;
- c) replacing said preadipocyte medium with a differentiation medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; a cyclic AMP inducer; 100 nM to 1  $\mu$ M insulin, or an equivalent amount of an insulin analogue; 16 nM to 1  $\mu$ M of a glucocorticoid; and a concentration of a PPAR $\gamma$  agonist effective to stimulate half-maximal differentiation of a human preadipocytes; and said compound in an appropriate vehicle or vehicle alone;
- d) incubating said cells at about 37°C for about 2-4 days;
- e) replacing said differentiation medium with an adipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; 100 nM to 1  $\mu$ M insulin, or an equivalent amount of an insulin analogue; 16 nM to 1  $\mu$ M of a glucocorticoid;
- f) incubating said cells at about 37°C for about one week and refeeding said cells at least once with the supplemented medium from step (e); and
- g) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with medium containing said compound;
- h) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with medium containing said vehicle alone; and
- i) comparing the number or percentage of differentiated cells from steps (g) and (h);

wherein said preadipocyte medium, said differentiation medium and said adipocyte medium are maintained at a pH of about 7.0 to 7.6 when in contact with said cells.

(3) A method for determining the ability of a compound to act as an insulin analogue, comprising:

- a) plating isolated human preadipocyte cells at a density of about 25,000 to 40,000 cells/cm<sup>2</sup> in a preadipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose;
- b) incubating said cells at about 37°C for about 4-24 hours until said cells are about 95-100% confluent;
- c) replacing said preadipocyte medium with a differentiation medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; a cyclic AMP inducer; 16 nM to 1 µM of a glucocorticoid; and a concentration of a PPAR $\gamma$  agonist or RXR agonist effective to stimulate differentiation of a human preadipocytes; and said compound in an appropriate vehicle or vehicle alone;
- d) incubating said cells at about 37°C for about 2-4 days;
- e) replacing said differentiation medium with an adipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; 16 nM to 1 µM of a glucocorticoid; and said compound in an appropriate vehicle or vehicle alone;
- f) incubating said cells at about 37°C for about 1-2 weeks and refeeding said cells at least every 3-4 days with said adipocyte medium; and
- g) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with medium containing said compound;
- h) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with medium containing said vehicle alone; and
- i) comparing the number or percentage of differentiated cells from steps (g) and (h);

wherein said preadipocyte medium, said differentiation medium and said adipocyte medium are maintained at a pH of about 7.0 to 7.6 when in contact with said cells.

4. A method for determining the ability of a compound to act as a glucocorticoid or glucocorticoid analogue, comprising:
- a) plating isolated human preadipocyte cells at a density of about 25,000 to 40,000 cells/cm<sup>2</sup> in a preadipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose;
  - b) incubating said cells at about 37°C for about 4-24 hours until said cells are about 95-100% confluent;
  - c) replacing said preadipocyte medium with a differentiation medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; a cyclic AMP inducer, 100 nM to 1 μM insulin, or an equivalent amount of an insulin analogue; and a concentration of a PPAR $\gamma$  agonist or RXR agonist effective to stimulate differentiation of a human preadipocytes; and said compound in an appropriate vehicle or vehicle alone;
  - d) incubating said cells at about 37°C for about 2-4 days;
  - e) replacing said differentiation medium with an adipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; 100 nM to 1 μM insulin, or an equivalent amount of an insulin analogue; and said compound in an appropriate vehicle or vehicle alone;
  - f) incubating said cells at about 37°C for about 1-2 weeks and refeeding said cells at least every 3-4 days with said adipocyte medium; and
  - g) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with medium containing said compound;
  - h) determining the number or percentage of differentiated cells in the cells from step (f) fed at step (c) with medium containing said vehicle alone; and
  - i) comparing the number or percentage of differentiated cells from steps (g) and (h);

wherein said preadipocyte medium, said differentiation medium and said adipocyte medium are maintained at a pH of about 7.0 to 7.6 when in contact with said cells.

(b) A method for introducing DNA into human adipocytes wherein said adipocytes are prepared using a method for differentiating human preadipocytes into adipocytes, comprising:

- a) plating isolated human preadipocytes at a density of about 25,000 to 30,000 cells/cm<sup>2</sup> in a preadipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose;
- b) incubating said cells at about 37°C for about 4-48 hours until said cells are about 95-100% confluent;
- c) replacing said preadipocyte medium with a differentiation medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose, 0.2 to 0.5 mM isobutylmethylxanthine; 100 nM to 1 μM insulin, or an equivalent amount of an insulin analogue; 16 nM to 1 μM of a glucocorticoid; and a concentration of a PPAR $\gamma$  agonist or RXR agonist effective to stimulate differentiation of human preadipocytes;
- d) incubating said cells at about 37°C for about 2-4 days;
- e) replacing said differentiation medium with an adipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; 100 nM to 1 μM insulin, or an equivalent amount of an insulin analogue; 16 nM to 1 μM of a glucocorticoid; and
- f) incubating said cells at about 37°C for about 1-2 weeks and refeeding said cells with said adipocyte medium at least every 3-4 days;

wherein said preadipocyte medium, said differentiation medium and said adipocyte medium are maintained at a pH of about 7.0 to 7.6 when in contact with said cells; and wherein said adipocyte is transiently or stably infected with at least one nucleic acid sequence.

(c) A method for identifying polypeptides secreted from cultured human adipocytes

prepared from preadipocytes using a method comprising:

- a) plating isolated human preadipocytes at a density of about 25,000 to 30,000 cells/cm<sup>2</sup> in a preadipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose;
- b) incubating said cells at about 37°C for about 4-48 hours until said cells are about 95-100% confluent;
- c) replacing said preadipocyte medium with a differentiation medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose, 0.2 to 0.5 mM isobutylmethylxanthine; 100 nM to 1 μM insulin, or an equivalent amount of an insulin analogue; 16 nM to 1 μM of a glucocorticoid; and a concentration of a PPAR $\gamma$  agonist or RXR agonist effective to stimulate differentiation of human preadipocytes;
- d) incubating said cells at about 37°C for about 2-4 days;
- e) replacing said differentiation medium with an adipocyte medium comprising a defined cell culture medium having or supplemented with 1.0-4.5 g/liter glucose; 100 nM to 1 μM insulin, or an equivalent amount of an insulin analogue; 16 nM to 1 μM of a glucocorticoid; and
- f) incubating said cells at about 37°C for about 1-2 weeks and refeeding said cells with said adipocyte medium at least every 3-4 days;

wherein said preadipocyte medium, said differentiation medium and said adipocyte medium are maintained at a pH of about 7.0 to 7.6 when in contact with said cells; and wherein said method comprises fractionating the polypeptides which are secreted.

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